NERL/MCEARD Publications

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Jan 1, 1999 - Dec 31, 1999

Presented Published

ABSTRCT/ORAL

Lindquist, H.D.A. Methods for detection of cryptosporidium sp. and giardia sp.. Presented at: ORSANCO Annual Meeting, Cincinnati, OH, October 21, 1999. — 10/21/1999

Contact: H. d. alan Lindquist

Abstract: There have been several waterborne outbreaks of giardiasis caused by infection with Giardia lamblia, and cryptosporidiosis, caused by infection with Cryptosporidium parvum. These outbreaks have created a need to detect these organisms in source and finished drinking water. The principal problems with detecting C. parvum and G. lamblia are their small size, their relatively low density in the water, and the difficulty of culturing these organisms from water samples. Hence, methods have been developed that rely on identification of low numbers of cysts and oocysts in large volume water samples. The initial attempt to quantify G. lamblia cysts and C. parvum oocysts involved filtration of the water sample, differential sedimentation, and identification of organisms by fluorescent antibody staining. This method is time consuming, has low recovery, especially of C. parvum oocysts, and it is difficult to confirm that cysts or oocysts are indeed cysts or oocysts from disease causing organisms, and that they may potentially cause disease. Newer methods have reduced the volume sampled, used different filtration media, and substituted immunomagnetic separation for differential sedimentation. Problems of identification and concentration have also been addressed by the introduction of flow cytometry, cell culture methods, genetic amplification methods, and fluorescent in situ hybridization.

Villamena, F.A., and de la Cruz, A.A. Caffeine-Imprinted Polymer: Its Application in the Detection of Non-Microbial Indicators of Human Fecal Contamination in Water. 1999 ASM Annual Meeting, Chicago, IL, 5/30-6/3/99. — 6/1/1999

Contact: Armah A. Delacruz-lynch

Abstract:

Villamena, F.A., and de la Cruz, A.A. Caffeine specificity of various non-imprinted polymers in aqueous media. Presented at: American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. — 8/23/1999

Contact: Armah A. Delacruz-lynch

Abstract: Limitations exist in applying the conventional microbial methods to the detection of human fecal contamination in water. Certain organic compounds such as caffeine, have been reported by the U.S. Geological Survey as a more suitable tracer. The employment of caffeine has been hampered by the absence of a rapid, sensitive, and inexpensive method to detect its presence in water. This may be rectified by the application of molecularly imprinted polymers. These polymers are used in molecular recognition, separation and sensor technology to detect specific molecules. Most studies involving molecular imprinting employ organic solvents which would limit its use for environmental monitoring of water quality. This paper focuses on the synthesis, characterization and binding studies of several caffeine-specific polymers in aqueous media. The polymer binding was optimized by varying the polymerization conditions such as the solvent, nature of initiation and type of cross-linker and functional monomer. One unexpected result was finding that some non-imprinted polymers exhibited comparable or greater binding capacity and selectivity for caffeine than the caffeine-imprinted polymers. The possible role of p-p interaction between the polymer and caffeine is discussed.

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Presented *Published*

Lindquist, H.D.A., Bennett, J.W., Broomall, K., Glover, G., and Schaefer, III, F.W. Counting cryptosporidium, an analysis of the utility of various cytometric techniques. Presented at: Annual Meeting of American Society of Parasitologists, Monterey, CA, July 5-10, 1999. 7/6/1999

Contact: H. d. alan Lindquist

Abstract: To develop, evaluate and implement methods to detect C. parvum oocysts in water, samples must be seeded with known concentrations of oocysts. Methods for counting oocysts are inaccurate and highly variable. To address this, several cytometric methods were tested: flow cytometry, solid phase cytometry, electric resistance particle characterization, hemacytometry, chamber slides, and epifluorecent well slide. These methods were compared on the basis of accuracy, variability, and practicality. One hundred oocysts were enumerated and sorted using the flow cytometer and directly deposited onto a 13 mm, 0.8 micron porosity membrane. For all other techniques, the oocyst suspension was counted and a volume calculated to contain 100 oocysts was delivered by pipette to this type of membrane. The membranes were stained with fluorescent conjugated antibody, and oocysts counted by epifluorescent microscopy. Other parameters influencing the selection of cytometric methods were recorded. Flow and solid phase laser cytometric methods were the most accurate; flow cytometry gave the most consistent results. Practical concerns may influence the selection of a method other than flow or solid phase cytometry. Electric resistance cytometry overestimates the concentration of oocysts due to its non-specific nature; this method also requires a large suspension volume. Hemacytometry overestimates the concentration of oocysts, but is useful for highly concentrated material. The epifluorescence well slide method is the most costly and most time consuming method based on microscopy. This method consistently underestimates the concentration of oocysts, has high variability, and is the least accurate of all methods. Where this method has been used for enumeration of material to test detection methods, the reported recovery overestimates the actual recovery potential, due to the underestimation of spike oocysts concentrations inherent with this method.

Bennett, J.W., and Lindquist, H.D.A. Epifluorescence microscopy and solid phase cytometry as confirmatory methods for the enumeration of protozoa by flow cytometry. Presented at: Annual Meeting of American Society of Parasitologists, Monterey, CA, July 5-10, 1999. 7/6/1999

Contact: H. d. alan Lindquist

Abstract: The detection of infective protozoan parasites contained in large volume environmental samples represents a unique challenge in environmental parasitology. Compounding this problem is the fact that infective stages of many protozoan parasites do not readily replicate in media or cell cultures without intensive effort. Since the infective dose for many protozoan parasites may be 1-10 protozoan cells there is a need for the development of methods to detect low numbers of protozoan infective stage organisms. Flow cytometry is an important tool for detection method development, because it provides the most accurate method of enumeration at lower concentrations of parasites. One humdred C. parvum oocysts can be enumerated and sorted with less than a 10% deviation between samples. However, a confirmation step must be done in order to be certain that the number of parasites sorted agrees with the number of parasites counted via the flow cytometer. In this study, several cytometric methods were used to enumerate C. parvum oocysts and giardia lamblia cysts. First, parasites were sorted onto a polycarbonate membrane based on their forward and side scatter properties using a flow cytometer. the sorted protozoa were then labeled with fluorescent antibodies. Solid phase cytometry and direct microscopic observation were then used as methods for confirming the counts obtained via flow cytometry. In theory, the use of solid phase cytometry provides an automated means to increase the accuracy of flow cytometry by eliminating both the possibility of over-counting as well as the likelihood of overlooking a field on the membrane. The results indicated, however, that there is no benefit to using solid phase cytometry when standard epifluorescence is available.

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Presented Published

Hester, J.D., Lindquist, H.D.A., Bobst, A.M., and Schaefer, III, F.W. A novel detection method for Encephalitozoon hellem in water. Presented at: International Symposium on Waterborne Pathogens, Milwaukee, WI, August 29-September 1, 1999. 8/30/1999

Contact: H. d. alan Lindquist

Abstract: Microsporidia are a ubiquitous group of protozoan pathogens, containing over 100 genera and hundreds of species. This group has been found in all animal groups. Recently, members of this group have been found even in man. The transmission state of all members of this group is a spore that is unique and diagnostic. Encephalitozoon hellem, a microsporidian that infects man, has been recovered from the central nervous system and eye. Encephalitozoon hellem is even known to disseminate throughout the human body to other sites like the urinary tract from where spores may be released to the environment. It can logically be concluded that there is potential for waterborne transmission of this pathogenic agent. Of all cases of waterborne gastroenteritis, only about fifty percent are attributable to a specific etiologic agent. Lack of specific, sensitive, and practical methods to identify pathogens in a matrix as complex as water contributes to this problem. Until adequate detection methods for these pathogens in water are developed, there is no way to know whether water is a significant route of transmission. The purpose of this research was to develop a method useful for the detection of E. hellem in water.Antibodies currently employed to detect human microsporidia spores have been shown to cross-react with other species not associated with pathogenicity in humans or not associated with human hosts at all. In order to overcome this problem with antibody specificity, a fluorescence in situ hybridization (FISH) technique for E. hellem spores was developed. The FISH technique employed a 6-carboxyfluorescein (6-FAM) labeled oligonucleotide probe targeting a specific sequence in the 18S ribosomal RNA (rRNA) of E. hellem. Samples of E. hellem spores, which have undergone FISH, are examined using a fluorescent microscope. Encephalitozoon hellem spores used in this study were cultured in Rabbit Kidney (RK-13:ATCC CCL-37) monolayers. Spores were purified from cell culture supernatants using Percoll buoyant density gradient centrifugation. Both transmission and scanning electron microscopy were used to insure quality and homogeneity of the spores produced in culture.The signal from purified FISH stained spores was bright enough to enable detection in reagent water and in Ohio River water concentrates as well. Preliminary specificity testing of the FISH probe in bacteria, algae, and protozoans found in environmental samples has shown no cross-reactivity. This is the first report of a FISH probe being used to label E. hellem spores specifically. There is some evidence that FISH technique maybe useful as a conservative indicator of viability, since rRNA within non-viable E. hellem spores should be degraded. Initial dual labeling employing an antibody against the spore surface in conjunction with the FISH probe has been shown possible. These data demonstrate the potential for using an antibody against the E. hellem spore coat for detection and using a FISH probe to confirm the identity of this clinically important microsporidian in environmental water.

Presented *Published*

Simmons, O.D., Juliano, J.J., Heaney, C.D., Francy, D.S., Schaefer, III, F.W., and Sobsey, M.D. Comparison of filtration methods for primary recovery of Cryptosporiidum parvum from water. Presented at: International Symposium on Waterborne Pathogens, Milwaukee, WI, August 29 - September 1, 1999. 8/30/1999

Contact: Frank W. Schaefer

Abstract: Waterborne disease outbreaks from contaminated drinking water have been linked to the protozoan parasite, Cryptosporidium parvum. To improve monitoring for this agent, the USEPA developed Method 1622 for isolation and detection of Cryptosporidium oocysts in water. Method 1622 is performance-based and involves filtration, concentration, immunomagnetic separation (IMS), antibody (FA) and counter dAPI) staining, and microscopic evaluation. Currently, a capsule filter system (1 micrometer nominal pore size, pleated, polethersulphone concentration of water samples by USEPA Method 1622 analysis of water samples.Four filtration systems were compared for primary concentration of C. parvum oocysts seeded into untreated environmental water samples: the capsule filter, a 1 micrometer absolute pore size, 293 mm diameter, track-etched, polycarbonate membrane filter disk, and two inexpensive, self-contained, disposable hollow fibers. Samples were subsequently processed by IMS, fluorescent antibody and DAPI stained, and microscopically evaluated, as outlined by Method 1622. Additionally, analyst proficiency in our laboratory was validated by performing precision and recovery experiments and method blanks with the capsule and membrane disk filters as directed in Method 1622. Cryptosporidium parvum oocyst recoveries from seeded 10-liter volumes of reagent water in the precision and recovery experiments were 31% (S.D. =10%) and 36% (S.D. = 11%) using the membrane disk and capsule filters, respectively.Replicate 10-liter field samples of raw waters, unspiked and laboratory spiked, were tested to evaluate the matrix effects from the environmental waters and to compare the recoveries achieved with the different primary concentration methods. Two analysts (O.D.S. III and C.D.H.) microscopically examined in the samples to ensure validity of the counts; oocysts identified in the unspiked field samples were confirmed by DAPI stain and Differential Interference Contrast microscopy. Oocyst recoveries from the environmental samples averaged 26% (S.D. = 25%) and 16% (S.D. = 6%) with the membrane disk and capsule filters, respectively, and 66% (S.D. = 5%) and 62% (S.D. = 6%) with the two hollow fiber ultrafilters, respectively. These results demonstrate the C. parvum oocysts can be recovered from environmental waters using the filters approved for use with Method 1622 but recoveries are lower and more variable than from reagent grade water. The disposable, hollow fiber ultrafilters recovered C. parvum oocysts more reliably and efficiently than two of the filters now recommended for Method 1622.

Kang, L.-T., and de la Cruz, A.A. Characteristic antibody responses during a waterborne cryptosporidiosis outbreak. Presented at: 1999 ASM Annual Meeting, Chicago, IL, May 30-June 3, 1999. 6/1/1999

Contact: Armah A. Delacruz-lynch

Abstract:

Detection of Waterborne Caliciviruses. Presented at: International Workshop on Human Caliciviruses, Atlanta, GA, March 29-31, 1999. 3/29/1999

Contact: G. shay Fout

Abstract:

Simmons, O.D., Heaney, C.D., Francy, D.S., Schaefer, III, F.W., and Sobsey, M.D. Recovery of Cryptosporidium parvum Oocysts from Environmental Surface Water Samples Collected Throughout the U.S. Using USEPA Method 1622. 1999 ASM Annual Meeting, Chicago, IL, 5/30-6/3/99. 6/1/1999

Contact: Frank W. Schaefer

Abstract:

Potter, B.B., Goldberg, M.M., and Clayton, C.A. The effects of multiple interferences on the EPA total cyanide method 335.4. Presented at: 1999 Industrial Wastewater Seminar, Greensboro, NC, October 5-6, 1999. 10/5/1999

Contact: Billy B. Potter

Abstract:

Presented *Published*

Rohrer, C., Berry, Jr., M.R., Akland, G.G., Roberts, M., and Pellizzari, E.D. Monitoring the transfer of pesticide residues from soft surfaces to foods. Presented at: AOAC International Meeting, Houston, TX, September 26-30, 1999. — 9/26/1999

Contact: Maurice R. Berry

Abstract:

Akland, G.G., Pellizzari, E.D., Hu, Y., Clayton, C.A., Long, K., Roberts, M., Berry, Jr., M.R., and Leckie, J. The three interacting factors associated with children's dietary exposures: environmental concentrations, food contamination, and children's behaviors. Presented at: American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. — 8/23/1999

Contact: Maurice R. Berry

Abstract: The dietary contribution to an aggregate exposure assessment is potentially an important pathway of exposure especially for young children. Enviornmental contamination appearing in the child's diet can result from contamination in the food as purchased or due to preparing, serving, handling, and/or eating food in the home. Food contamination while eating is of special concern for children 1-3 years old, since this age group has a high frequency of hand to food, hand to surface, and surface to food interactions. These sources of food contamination may be very important in homes with high surface concentrations of chemical residue which may have resulted from outdoor air, track-in of particles, and/or indoor sources. Results of a study designed to evaluate the sources and amount of dietary lead exposure for low socioeconomic status children living in lead contaminated homes in the Newark, New Jersey area will be presented.

Wei, X., Shoemaker, J.A., Gallagher, P.A., Schwegel, C.A., and Creed, J.T. Extraction and identification of arsenosugars in commercially available seaweeds. Presented at: International Ion Chromatography Symposium, San Jose, CA, September 12-15, 1999. — 9/13/1999

Contact: John T. Creed

Abstract: Arsenosugars, mostly in the form of dimethylarsinylribosides, are widely found in marine plants. Since the first arsneosugar was identified in 1982, fifteen arsenosugars have been isolated and identified as algal constituents. Seaweed has been a popular dietary food in Asian Pacific culture for ages, however, the metabolic fate of arsenosugars remains virtually an unknown. Preliminary toxicological studies showed that arsenosugars are much less toxic than inorganic arsenics while arsenic speciation studies on human urine after algea ingestion suggested that arsenosugars experienced certain metabolic changes and produce DMA as well as a few other unknown arsenic metabolites. From an exposure assessment perspective the arsenosugars and the associated metabolites are potential false positives in a urinary arsenic exposure assessment. The first step in evaluating the impact of arsenosugars on a urinary arsenic exposure assessment method is to produce a semi-purified standard material and characterize it chromatographically and structurally.In this study, three arsenosugars, identified via ion chrmatography and hydride generation ICP-MS, were extracted from two commercial seaweeds using Accelerated Solvent Extraction. The extracts were then treated with C18 and fraction collected using a preparatory PRPX-100 column. These crude extracts (containing individual arsenosugars) were then characterized chromatographically using IC-ICP-MS and structurally using IC-electrospray MS/MS.

Rohrer, C., Berry, Jr., M.R., Akland, G.G., Roberds, M., and Pellizzari, E.D. Transfer of pesticides from surfaces to foods for the estimation of dietary exposure to children. Presented at: American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. — 8/23/1999

Contact: Maurice R. Berry

Abstract:

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Presented *Published*

Berry, Jr., M.R., Melnyk, L.J., Rohrer, C., Akland, G.G., Clayton, C.A., Hu, Y., Aragon, E.D., Roberds, J.M., and Pellizzari, E.D. Measuring dietary exposure of young children. Presented at: American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. — 8/23/1999

Contact: Maurice R. Berry

Abstract: Young children do not consume foods in a structured manner. Their foods contact surfaces (hands, floors, eating surfaces, etc.) that may be contaminated while they are eating them. Thus, dietary exposures of young children are difficult to accurately assess or measure. A recent study on dietary exposure of children to lead has begun to explore potential pathways of dietary contamination, and ways to measure them, and has shown intakes may potentially increase by a factor of four when foods are handled by the child in a contaminated environment. Similar excess exposures of children to pesticides may also be possible in residential and daycare environments, and improved assessment and measurement techniques are needed to support the aggregate exposure assessments required by the Food Quality Protection Act of 1996. The purpose of this research is to develop procedures to measure the daily, dietary intake of a 1-3 year old child. Since this age group has a tendency to excessively handle their foods, fingering items on eating surfaces and dropping food on the floor while eating, they are at a higher risk from dietary exposure to the pesticides which contaminate their environment.

Rohrer, C., Berry, Jr., M.R., Roberds, M., Akland, G.G., and Pellizzari, E.D. Monitoring the transfer of pesticide residues from soft surfaces to foods. Presented at: AOAC International Meeting, Houston, TX, September 26-30, 1999. — 9/27/1999

Contact: Maurice R. Berry

Abstract: The Food Quality Protection Act of 1996 requires assessing pesticide residue exposures to children. Contact of soft surfaces by foods and the ingestion of the food represents a pesticide exposure pathway. Chlorpyrifos has widespread use indoors and would be protected from environmental degradation by rain, sun or temperature fluctuations. Pesticides are known to accumulate on surfaces after indoor pesticide application. Children's exposure to pesticides from surfaces such as napkins and tablecloths is related to residue amounts present on surfaces prior to contact, the foods contacted and their transfer coefficient. Surfaces contaminated with pesticides were evaluated for transferable residues by pressing with C-18 disks, swabbing the surface with isopropanol wipes, and contacting with foods. GC/MS was utilized for quantitation of pesticides. Average transfers of chlorpyrifos from tablecloth to bologna were 8.2% and 8.7%, respectively, with and without 3.5 g/cm2 applied pressure. These data suggest pesticides are transferred to foods ingested by children. However, significance of exposure will depend on food type, time of contact, frequency and pressure.

Melnyk, L.J., Berry, Jr., M.R., Sheldon, L.S., Freeman, N., and Pellizzari, E.D. Dietary exposure of children to lead. Presented at: American Chemical Society National Meeting, New Orleans, LA, August 22-26, 1999. 8/23/1999

Contact: Maurice R. Berry

Abstract: Children are the most susceptible population to lead exposure because 1) they have more opportunity for contact with lead sources due to their activities, 2) lead adsorption occurs more readily in a child as compared to an adult, and 3) the child's development is more vulnerable to lead than adults. Low levels of lead in the blood have been shown to cause adverse health effects: the level of concern for children is currently 10 ug/dL. The contribution of dietary exposure of lead to increased blood lead levels is not well characterized and is becoming a larger portion of exposure as others are decreasing (e.g., from leaded gasoline, lead in pain, lead solder in food cans, etc.). This study was conducted with experimental techniques to obtain estimated dietary lead intakes of children 2 to 3 years of age who live in homes contaminated with environmental lead. General research objectives were (1) to identify and quantify the sources of idetary lead exposure, (2) to estimate potential lead intakes for children consuming food in contaminated environments, and (3) to investigate potential correlations between daily exposure and measured blood levels of lead.Dietary exposure was evaluated by collecting food samples that were representative of the foods the young children who participated in the study ate in their homes. A 24-hr. duplicate of all foods plus sentinel foods, i.e., individual food itmes used to represent foods for expsoure during handling, were collected from 48 children. Seven of the participants were revisited three times and three participants were revisited once to obtain information on the variation in dietary intakes. Drinking water was evaluated both as part of the beverage sample and by itself. Additional information collected included lead concentrations from hand wipes, floor wipes, and venous blood; and questionnaire responses on activities related to exposure.All samples were analyzed by inductively coupled plasma mass spectrometry (ICP/MS) at Research Triangle Institute, NC. Measurements indicated that the activities and hygiene practices of children and their environment can influence the amount of lead ingested with food, which must be taken into consideration when detemining total dietary intake for the the defined subpopulation. Estimated dietary intakes of lead (37.5 ug Pb/day) were >4 times the measured 24-hr. duplicate-diet results (8.27 ug Pb/day), which were almost six times higher than the currently reported estimates from national survey (1.40 ug/day). Statistically significant correlations were established ($p<0.05$) between blood lead levels and hand wipes, blood levels and apples contacting contaminated surfaces, hand wipes and bananas contacting contaminated hands, and between drinking water and solid foods. This study indicates that the dietary route of exposure to lead is impacted by eating activities of children living in a lead contaminated environment and that excess dietary exposures are occurring.

Shoemaker, J.A., and Magnuson, M.L. Application of solid phase microextraction GC/MS to the characterization of hydrophilic disinfection by-products in water. Presented at: 1999 ASMS Conference, Dallas, TX, June 13-17, 1999. 6/14/1999

Contact: Jody A. Shoemaker

Abstract: The U.S. Environmental Protection Agency has given high priority to research aimed at developing methods to extract hydrophilic disinfection by-products (DBPs) from drinking water. Public water supplies are treated with a variety of chemicals aimed at reducing or eliminating infectious diseases. Chlorine is the most common disinfectant used to combat waterborne microbial diseases; however, the use of ozone, chlorine dioxide, and chloramine as disinfectants in on the rise. While reducing the microbial risk, the use of these disinfectants poses a new potential risk due to DBPs formed during the water treatment process. Over the years, analytical techniques such as liquid-liquid or liquid-solid extraction followed by gas chromatography/mass spectrometry (GC/MS) analysis have been employed to characterize DBPs in drinking water. These techniques are suitable for detecting the less water soluble, semivolatile DBPs. However, a large portion of the hydrophilic fraction of drinking water is not being extracted with these conventional methods. Our research is now aimed at using solid phase microextraction (SPME) GC/MS to extract DBPs from model humic acid solutions treated with chlorine and monochloramine.

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Presented *Published*

Berry, Jr., M.R., Rohrer, C., Melnyk, L.J., Akland, G.G., Clayton, C.A., Hu, Y., Aragon, E.D., Roberds, M., and Pellizzari, E.D. Measuring dietary exposure of young children. Presented at: American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. — 8/23/1999

Contact: Maurice R. Berry

Abstract: Young children do not consume foods in a structured manner. Their foods contact surfaces (hands, floors, etc.) that may be contaminated with pesticides. Thus, dietary exposure measurements of young children are difficult to measure, but are needed to support the aggregate exposure assessments required by the Food Quality Protection Act of 1996. A protocol was developed to measure the daily, dietary intacke of a 1-3 yr child. Procedures which supplement duplicate-diet sampling provide input parameters to a model developed to predict total dietary intake. The procedures involve video taping of eating activities, concentration measurements and transfer factors for surfaces, and surrogate sampling of foods handled by the child. Video analysis of 10 children determine surface, food and hand contact frequencies and duration and surface transfer coefficients were measured. Field testing is being conducted to measure dietary intakes of pesticides by young children.

Kauffman, P., and Morgan, J.N. Determination of organophosphate pesticides in composite diet samples. Presented at: AOAC International Meeting, Houston, TX, September 26-30, 1999. — 9/27/1999

Contact: Jeffrey N. Morgan

Abstract: USEPA's National Exposure Research Laboratory conducts research to measure the exposure of individuals to chemical pollutants through the diet, as well as other media. In support of this research, methods are being evaluated for determination of pollutants, including organophosphate pesticides, in composite diet samples. Existing methods for pesticides generally have been developed for regulatory purposes and often do not have sufficiently low detection limits for exposure studies. Consequently, there is a need to improve the performance of these analytical methods for the determination of pesticides in composite diet samples. Diatomaceous earth and C18 rewversed phase chromatography have been utilized by researchers at the U.S. FDA as a media for the cleanup of five organophosphate pesticides in solutions of edible vegetable oils. The current study evaluated this cleanup technique as applied to high fat (10%) and low fat (1%) composite diet samples, fortified with thirty-seven organophosphate pesticides. Samples were extracted with a methylene chloride-acetone solvent mixture using accelerated solvent extraction and cleaned up using the aforementioned media. Pesticides were quantitated by capillary GLC using a DB-17 column with flame photometric detection (phosphorous mode). Results of this study demonstrated that the procedure separated the pesticides from greater than 95% of the lipid material. The fortified pesticide recoveries range from 30 to 87%.

Morgan, J.N., Akinbo, O., Fernando, R., and Pellizzari, E.D. Determination of metals in composite diet samples by ICP-MS. Presented at: AOAC International Meeting, Houston, TX, September 26-30, 1999. — 9/27/1999

Contact: Jeffrey N. Morgan

Abstract: In order to assess an individual's toal exposure to contaminants in the environment, it is essential that the contribution of dietary exposure be quantified. As a result, USEPA's National Exposure Research Laboratory has initiated a program to develop methods to measure chemical pollutants in dietary samples collected from individuals. Previous efforts have utilized ICP-AES and GFAAS techniquesw for determination of metals in composite diets. However, there is often a trade-off between sensitivity and sample throughput with these techniques. ICP-MS offers sensitivity comparable to or better than GFAAS while retaining sample throughput comparable to ICP-AES. This study evaluated the applicability of ICP-MS techniques to determination of metals in composite diets. An ICP-MS method for determination of Al, Ba, Cd, Cr, Cu, Pb, Mn, Ni, V and Zn is presented. The procedure utilizes atmospheric pressure microwave digestion to solubilize analytes in homogenized food samples followed by ICP-MS analysis. Recovery of certified elements from SRMs ranged from 92-119% with rSD ranging from 0.4 - 1.9%. Recovery of elements from fortified composite diet samples ranged from 75-129% with RSDs ranging from 0 - 11.3%. MDLs ranged from 1 -1733 ppb, with high values due to significant amounts of certain elemnts naturally present in foods. Results of this study demonstrate that low resolution ICP-MS provides precise and accurate measurement of the elements tested in composite diet samples.

Presented *Published*

Raymer, J.H., Pellizzari, E.D., and Shoemaker, J.A. Analytical methods for water disinfection by-products in foods and beverages. Presented at: American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. 8/23/1999

Contact: Jody A. Shoemaker

Abstract: The determination of exposure to drinking water disinfection byproducts (DBPs) requires an understanding of how drinking waters come into contact with the human through multiple pathways. The most significant pathway is the ingestion of drinking water. However, ingestion can occur as direct ingestion of the water or as a result of the interaction of the DBPs in tap water with foods. Consumption surveys indicate that approximately 2/3 of the drinking water ingested is through other sources, e.g., frozen juices, coffee, etc.In order to facilitate the investigation of human exposure to DBPs, method development efforts were initiated for haloactonitriles, halo ketones, and chloropicrin (EPA Method 551.1 analytes) and the haloacetic acides (EPA Method 552.2 analytes) in food and beverages. The recoveries of the target analytes were investigated both from composite foods and beverages in addition to individual foods and beverages selected based on their water content and frequency of consumption. The 551.1 analytes were generally well-recovered from beverages following extraction with methyl-t-butyl ether while the addition of acetone was found to be needed for foods. The 552.2 analytes were generally well-recovered from foods but some were problematic from beverages. In both cases, certain matrices were more problematic than others with regard to processing and analyte recovery. Additional clean-up steps were developed and integrated into the methods to overcome the complexity of the matrices.

Raymer, J.H., Pellizzari, E.D., and Shoemaker, J.A. Human exposure to water disinfection by-products via foods and beverages. Presented at: American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. 8/23/1999

Contact: Jody A. Shoemaker

Abstract: The ingestion of tap water is a major route of exposure to water disinfection byproducts (DBPs), including haloacetonitriles, haloketones, and haloacetic acids. A potentially significant alternate route of exposure is through the consumption of beverages prepared with tap water and through foods containing or prepared using tap water that contains DBPs. Using analytical methods developed during this project, controlled experiments were designed and conducted to study the potential for uptake of DBPs into foods prepared using tap water. Foods such as condensed soups, rice, pasta, beans, meat, and fresh vegetables were prepared using reagent water spiked to contain the target DBPs at realistic concentrations. The uptake of DBPs into the foods was evaluated and a mass balance was conducted to help understand the transfer mechanisms.

Melnyk, L.J., Berry, Jr., M.R., Sheldon, L.S., Freeman, N., and Pellizzari, E.D. Dietary exposure of children to lead. Presented at: American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. 8/23/1999

Contact: Maurice R. Berry

Abstract: Childen are the most susceptible population to lead exposure because 1) they have more opportunity for contact with lead sources due to their activities, 2) lead absorption occurs more readily in a child as compared to an adult, and 3) the child's development is more vulnerable to lead than adults. Low levels of lead in the blood have been shown to cause adverse health effects: the level of concern for children is currenlty 10 ug/dL. The contribution of dietary exposure of lead to increased blood lead levels is not well characterized and is becoming a larger portion of exposure as others are decreasing (e.g., from leaded gasoline, lead in paint, lead solder in food cans, etc.). Dietary exposure was evaluated by collecting food samples that were representative of the foods the young children who participated in the study ate in their homes. A 24-hr duplicate of all foods plus sentinel foods, i.e., individual food items used to represent foods for exposure during handling, were collected from 48 children. Seven of the participants were revisited three times and three participants were revisited once to obtain information on the variation in dietary intakes. Drinking water was evaluated both as part of the beverage sample and by itself. Additional information collected included lead concentrations from hand wipes, floor wipes and venous blood; and questionnaire responses on activities related to exposure. A general conclusion is that, for childen in this study, food eaten and handled in a lead contaminated environment increased dietary exposure to lead.

Presented Published

Rohrer, C., Berry, Jr., M.R., Akland, G.G., Roberds, M., and Pellizzari, E.D. Transfer of pesticides from surfaces to foods for the estimation of dietary exposure of children. Presented at: American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. 8/23/1999

Contact: Maurice R. Berry

Abstract: Since small children spend much of their time in contact with contaminated surfaces, residues of pesticides found on floors and other surfaces contribute to their aggregate exposures. Any dislodgeable pesticide residues on hands and surfaces may be transferred to foods while being consumed by the child contributing to increased dietary intakes. This study evaluated pesticde levels transferred to food during contact with surfaces. Results indicate average transfers of 6% to 10% of diazinon, malathion, chlorpyrifos, isofenphos, heptachlor and permethrins to bologna after 10 minute contact with hardwood and ceramic tile. Other food and surface combinations yielded similar low level transfers of pesticides.

Gallagher, P.A., Schwegel-Brockhoff, C.A., Shoemaker, J.A., Creed, J.T., McKiernan, J.W., and Wei, X. Accelerated solvent extraction of arsenicals in seaweed with ion chromatography separation and ICP-MS detection. Presented at: 217th ACS National Meeting, Anaheim, CA, March 21-25, 1999. 3/22/1999

Contact: Jody A. Shoemaker

Abstract:

Shoemaker, J.A., and Magnuson, M.L. Application of solid phase microextraction GC/MS to the characterization of hydrophilic disinfection by-products in water. Presented at: 1999 ASMS Conference, Dallas, TX, June 13-17, 1999. 6/14/1999

Contact: Jody A. Shoemaker

Abstract:

Dufour, A.P. Predicting bather risk with microbial indicators of water quality--can the current approach be improved?. Presented at: Watershed 99 Water, Environment & Health Conference, Guildford, UK, June 21-24, 1999. 6/22/1999

Contact: Alfred P. Dufour

Abstract:

Dufour, A.P. Risk and Risk Assessment in Water-Based Recreation. Presented at: WHO Public Health Seminar - Recreational Waters: Risks & Benefits, Rome, Italy, February 19, 1999. 2/19/1999

Contact: Alfred P. Dufour

Abstract: The great number of individuals using recreational water resources presents a challenge with regard to protecting the health of these recreationists. Risk assessment provides a framework for characterizing the risk associated with exposure to microbial hazards and for managing recreational water resources. This presentation will review the process of risk assessment as it rlates to microbial hazards associated with recreational waters. The process includes hazard identification, effects assessment, exposure assessment and risk characterization. Examples relevant to each step in the process will be discussed. This process will lead to rational decision making by risk manager, who have the ultimate responsibility for maintaining safe recreational waters.

Jan 1, 1999 - Dec 31, 1999

Presented Published

Krishnamurthy, T., Nair, H., Jabbour, R., Richardson, S.D., Kryak, D.D., Ware, M.W., and Schaefer, III, F.W. Species and genus differentiation of parasites (Giardia and Cryptosporidium) by MALDI - mass spectrometry. Presented at: International Symposium on Waterborne Pathogens, Milwaukee, WI, August 29 - September 1, 1999. 8/30/1999

Contact: David D. Kryak

Abstract: The protozoan parasites, Cryptosporidium parvum and Giardia lamblia, have been responsible for numerous waterborne outbreaks of gastrointestinal illness in the United States. The 1993 cryptosporidiosis outbreak in Milwaukee affected approximately 400,000 people and resulted in over 100 deaths. Both Cryptosporidium parvum oocysts and Giardia lamblia cysts have been found in many surface waters and are generally resistant to treatment with the chemical disinfectants used by water utilities. At the time of the Milwaukee outbreak, the accepted method for measuring Cryptosporidium in water, beside being extremely labor-intensive, could not distinguish between viable and non-viable (or infective vs. non-infective) organisms. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) recently has shown promise for identifying bacteria and distinguishing between species. We conducted MALDI-MS investigations to determine if MALDI-MS can be used to identify pathogenic protozoans and also to determine if live organisms can be distinguished from dead ones.The acquired spectrum of each of the organisms contained numerous protein marker ions, and the observed noise level was low. Careful inspection of MALDI-mass spectra of both pathogens revealed numerous marker proteins with molecular masses ranging from 3-36 kDa. Mass spectra of Giardia lamblia and Giardia muris cysts reveqled common biomarkers at 6662, 10365, 10571, 11321, and 12446 kDa, which may be indicators for the genus Giardia. The marker proteins (>20) observed in the MALDI spectrum of Giardia lamblia, ranging from 3-36 kDa, may be representative of the lamblia species. Similarly, proteins (25; 3-32 kDa) detected only in the spectrum of Giardia muris may be representative of the muris species. Similar marker proteins for Cryptosporidium parvum and Cryptsporidium muris oocysts were also found, indicating that these species may be distinguished.The solutoins could be stored at 40 degrees C, at least over a period of two days, without noticeable decomposition. The spectral data could be generated reproducibly, and observed detection limits for these mircoorganisms were also significantly low (~10 to 100 (oo)cysts). When lyophilized organisms were suspended in PBS buffer and/or detergents, the observed signals were diminished either significantly or totally. However, clean up of such samples over a reverse-phase cartridge (C8) nullified the ionization suppression caused by the salts and detergents. Investigations are presently underway to determine the reproducibility of the generated spectral data and the effect of variation in growth conditions and sample preparation on observed biomarkers. Similarly, sample concentration techniques are being developed to allow the detection of low levels of these organisms in larger volumes of water.

Presented *Published*

Krishnamurthy, T., Nair, H., Jabbour, R., Richardson, S.D., Kryak, D.D., Ware, M.W., and Schaefer, III, F.W. MALDI-MIS investigations of drinking water pathogens--Giardia and Cryptosporidium. Presented at: 47th ASMS Conference on Mass Spectrometry & Allied Topics, Dallas, TX, June 13-17, 1999. — 6/14/1999

Contact: David D. Kryak

Abstract: The protozoan parasites, Cryptosporidium parvum and Giardia lamblia, have been responsible for numerous waterborne outbreaks of gastrointestinal illness in the United States. The 1993 cryptosporidiosis outbreak in Milwaukee affected approximately 400,000 people and resulted in over 100 death. Both Cryptosporidium parvum oocysts and Giardia lamblia cysts have been found in many surface waters and are generally resistant to treatment with the chemical disinfectants used by water utilities. At the time of the Milwaukee outbreak, the accepted method for measuring Cryptosporidum in water, beside being extremely labor-intensive, could not distinguish between viable and non-viable (or infective vs. non-infective) organisms. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) recently has shown promise for identifying bacteria and distinguishing between species. We conducted MALDI-MS investigations to determine if MALDI-MS can be used to identify pathogenic protozoans and also to determine if live organisms can be distinguished from dead ones.The acquired spectrum of each of the organisms contained numerous protein marker ions, and the observed noise level was low. Careful inspection of MALDI-mass spectra of both pathogens revealed numerour marker proteins with molecular massdes ranging from 3-36 kDa. Mass spectra of Giardia lamblia and Giardia muris cysts revealed comon biomarkers at 662, 10365, 10571, 11321, and 12446 kDa, which may be indicators for genus Giardia. The marker proteins (>20) observed in MALDI spectrum of Giardia lamblia, ranging rom 3-36 kDa, may be representative of the lamblia species. Similary, proteins (>25; 3-32 kDa) detected only in the spectrum of Giardia muris may be representative of the muris species. Similar marker proteins for Cryptosporidium parvum and Cryptosporidium muris oocysts were also found, indicating that these species may be distinguished.The solutions could be stored at 4 degrees C, at least over a period of two days, without noticeable decomposition. The spectral data could be generated reproducibly, and the observed detection limits for these microorganisms were also significantly low (~10 to 100 (oo)cysts). When the lyophilized organisms were suspended in PbS buffer and/or detergents, the observed signals were diminished either significantly or totally. However, clean up of such samples over a reverse phase cartridge (C8) nullified the ionization suppression caused by the salts and detergents. Investigations are presently underway to determine the reproducibility of the generated spectral data and the effect of variation in growth conditions and sample preparation on observed biomarkers. Similarly, sample concentration techniques are being developed to allow the detection of low levels of these organisms in larger volumes of water.

Vesper, S.J., Haugland, R.A., Dearborn, and Sorenson, W.G. Characterization of Stachybotrys chartarum Strains from the Cleveland Pulmonary Hemosiderosis Outbreak. Presented at: 1999 ASM Annual Meeting, Chicago, IL, May 30-June 3, 1999. 1999. — 6/1/1999

Contact: Stephen J. Vesper

Abstract:

EPA PROCEED

Lindquist, H.D.A. "Emerging pathogens of concern in drinking water.", 1999. — 8/3/1999

Contact: H. d. alan Lindquist

Abstract:

Cross, M. Data quality objectives and statistical design support for development of a monitoring protocol for recreational waters. Presented at: Data Quality Objectives, Cincinnati, OH, June 14-16, 1999. — 6/14/1999

Contact: Kristen P. Brenner

Abstract:

Jan 1, 1999 - Dec 31, 1999

Presented Published

JOURNAL

Bennett, J.W., Gauci, M., Lemoenic, S., Schaefer, III, F.W., and Lindquist, H.D.A. A comparison of enumeration techniques for cryptosporidium parvum oocysts. Journal of Parasitology 85 (6):1168-1170 (1999). EPA/600/J-00/131. — 12/1/1999

Contact: Frank W. Schaefer

Abstract: A variety of methods have been used to enumerate Cryptosporidium parvum oocysts from source or drinking waters. The reliability of these counting methods varies, in part, with suspension density, sample purity, and other factors. Frequently, the method of determination of suspension density is not reported by authors. To confound the problem, each method of counting has large inherent variation. There is a relationship between suspension density, overall number of organisms counted, and counting mechanism accuracy that should be accounted for when selecting a counting mechanism. This study selected a maximum acceptable coefficient of variatin (CV) to be 10%. A method was considered unreliable if this standard was not achieved. Flow cytometry achieved this standard at 486 oocysts/ml. Counting with a Coulter counter achieved this level of reliability at about 1,230 oocysts/ml. Neither chamber slides nor fluorescent antibody-stained well slides ever demonstrated less than 10% CV. However, estimates of the minimum required concentrations were 5,100 oocysts/ml and approximately 6,500 oocysts/ml, respectively. The hemacytometer provided counts accurate to a 10% CV at a concentration of at least 60,000 organisms/ml. Of the methods tested, flow cytometry provided the least amount of variability at low suspension densities.

Shoemaker, J.A., Munch, J.W., and Behymer, T.D. Evaluation of solid phase microextraction for the analysis of hydrophilic compounds. 1999. Journal of Exposure Analysis and Environmental Epidemiology (Stockton Press) 9 (3):181-191 (1999). — 3/1/1999

Contact: Jody A. Shoemaker

Abstract:

McKiernan, J.W., Creed, J.T., Schwegel, C.A., Caruso, J.A., and Lorenzana, R.M. A comparison of automated and traditional methods for the extraction of arsenicals from fish. Journal of Analytical Atomic Spectrometry 14:607-613 (1999). EPA/600/J-01/009. — 10/1/1999

Contact: John T. Creed

Abstract:

Evans, O.M. On-line deoxygenation in reductive (and oxidative) amperometric detection: environmental applications in the liquid chromatography of organic peroxides. Analyst (The Royal Society of Chemistry) (124):1811-1816 (1999). EPA/600/J-01/030. — 12/31/1999

Contact: Otis M. Evans

Abstract:

Haugland, R.A., Vesper, S.J., and Wymer, L.J. Quantitative measurement of Stachybotrys chartarum Conidia using real time detection of PCR products with the TaqMan fluorogenic probe system. Molecular and Cellular Probes (Academic Press) 13 (5):329-340 (1999). EPA/600/J-01/032. — 10/1/1999

Contact: Richard A. Haugland

Abstract:

SYMPOS/CONF

Hester, J.D., Lindquist, H.D.A., Bobst, A.M., and Schaefer, III, F.W. A novel detection method for Encephalitozoon hellem in water. Presented at: International Symposium of Waterborne Pathogens, Milwaukee, WI, August 29 - September 1, 1999. — 8/30/1999

Contact: Frank W. Schaefer

Abstract:

Jan 1, 1999 - Dec 31, 1999

Presented Published

Rodgers, M.R., Shadix, L., and Feige, M.A. Occurrence of aeromonas bacteria in potable waters. Presented at: International Symposium of Waterborne Pathogens, Milwaukee, WI, August 29 - September 1, 1999. 8/30/1999

Contact: Mark R. Rodgers

Abstract: